

CLAIMS

What is claimed is:

1. A particle comprising:
 - 5 a) a fluorescent analyte detection dye, the analyte detection dye being capable of being excited by light at a first excitation wavelength and capable of emitting light at a maximum wavelength when excited, and
 - b) two or more than two fluorescent labels in a first combination of relative amounts, the fluorescent labels being capable of being excited by light of a same second
10 excitation wavelength and capable of emitting lights at maximum wavelengths, distinguishable from each other, respectively, wherein
the maximum wavelength of the emitted light of the analyte detection dye is different from the first and second maximum wavelengths of the emitted lights of the fluorescent labels by at least 100 nm, and
15 the first and second excitation wavelengths differ by at least 100 nm and one of the excitation wavelengths is greater than about 750 nm.
2. The particle of claim 1 wherein the analyte detection dye is externally complexed to the outside of the particle, and the fluorescent labels are embedded within the particle.
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3. The particle of claim 1 wherein the fluorescent labels are both cyanine dyes having emitting lights greater than 750nm.
4. The particle of claim 1 wherein light at the first excitation wavelength causes
25 substantially no emitted light by the fluorescent labels and light at the second excitation wavelength causes substantially no emitted light by the analyte detection dye.
5. The particle of claim 1 wherein the first excitation wavelength is less than 750 nm.
- 30 6. The particle of claim 1 wherein the second excitation wavelength is greater than 750

nm.

7. The particle of claim 1 wherein the first excitation wavelength is one of about 530 nm, about 630 nm, or about 650 nm.

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8. The particle of claim 1 wherein the second excitation wavelength is about 780 nm.

9. The particle of claim 1 wherein the maximum intensity of the first wavelength differs from the maximum intensity of the second wavelength by at least 20 nm.

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10. The particle of claim 1 further comprising a second analyte detection dye.

11. The particle of claim 1 further comprising

15 a second particle having a second fluorescent analyte detection dye, the second fluorescent analyte detection dye being capable of being excited by light at an excitation wavelength and capable of emitting light at a maximum wavelength when excited, and two or more than two fluorescent labels in a second combination of relative amounts, wherein

each fluorescent label is capable of being excited by light of the same second excitation wavelength and capable of emitting light a maximum wavelengths,
20 distinguishable from each other respectively, and

the maximum wavelength of the emitted light of each fluorescent analyte detection dye is different from the maximum wavelengths of the emitted lights of each of the fluorescent labels by at least 100 nm, and

the excitation wavelength of each fluorescent analyte detection dye differs by at least
25 100 nm from the excitation wavelength of each of the fluorescent labels, and one of the excitation wavelengths is greater than about 750 nm.

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12. The particles of claim 11 wherein the fluorescent labels are present in the first and second particles in predetermined amounts.

13. The particles of claim 11 wherein the combination of relative amounts of fluorescent label in each particle is different.
14. The particles of claim 11 wherein the first particle has a first size and the second
5 particle has a second size and the first and second particles are each capable of emitting scattered light when illuminated, wherein the scattered light of the first particle is different than the scattered light of the second particle.
15. The particles of claim 11 wherein the first fluorescent analyte detection dye and the
10 second fluorescent analyte detection dye can be excited by light of the same wavelength.
16. The particles of claim 11 wherein the first fluorescent analyte detection dye and the second fluorescent analyte detection dye can be excited by light of different wavelengths.
- 15 17. The particles of claim 11 further comprising a second analyte detection dye.
18. An analyte detection system comprising:
- a) one or more than one particle, each particle comprising a fluorescent analyte detection dye capable of being excited by light at an excitation wavelength and capable of
20 emitting light when excited at a maximum wavelength, and two or more than two fluorescent labels in a combination of relative amounts, wherein each fluorescent label is capable of being excited by light of a same excitation wavelength and capable of emitting light when excited at maximum wavelengths; distinguishable from each other, respectively, and
- 25 the maximum wavelength of emitted light of each fluorescent analyte detection dye is different from the maximum wavelength of emitted light of each of the fluorescent labels by at least 100 nm, and
- the excitation wavelength of each analyte detection dye differs by at least 100 nm from the excitation wavelength of each of the fluorescent labels and one of the excitation
30 wavelengths is greater than about 750 nm.

- b) means for exciting the fluorescent dye;
 - c) means for exciting the first and second fluorescent labels;
 - d) means for detecting the emitted lights; and
 - 5 e) means for correlating the detected emitted lights with a particular particle under analysis.
19. The analyte detection system of claim 18 comprising more than one particle wherein the combination of relative amounts of fluorescent label in each particle is different.
- 10 20. The analyte detection system of claim 18 comprising more than one particle wherein the particles are of different size and including means for illuminating the particles to generate scattered lights, means for detecting the scattered lights, and means for correlating the detected emitted lights and the scattered lights with the particle under analysis.
- 15 21. An assay system comprising a particle having:
- a) a fluorescent analyte detection dye capable of being excited by light at a first excitation wavelength and capable of emitting light when excited;
 - b) two or more than two fluorescent labels, each fluorescent label being capable of being excited by light of a same second excitation wavelength and capable of emitting light
 - 20 when excited at maximum wavelengths, distinguishable from each other, respectively;
 - c) a first receptor; and
 - d) an analyte, wherein
- the analyte, first receptor, and the fluorescent analyte detection dye form a fluorescent complex on the particle, and
- 25 the emitted light of the fluorescent analyte detection dye is different from the wavelengths of emitted lights of each of the fluorescent labels by at least 100 nm, and the first and second excitation wavelengths differ by at least 100 nm and one of the excitation wavelengths is greater than about 750 nm.
- 30 22. The assay system of claim 20 further comprising a second receptor, the first

receptor, the analyte and the second receptor forming a fluorescent complex on the particle.

23. A method for detecting an analyte on a particle comprising:

- a) moving one or more than one particle through an examination zone, each particle
5 having a fluorescent analyte detection dye, and two or more than two fluorescent labels;
- b) directing an exciting light of a first wavelength at each particle in the
examination zone;
- c) directing an exciting light of a second wavelength at each particle in the
examination zone, wherein the fluorescent analyte detection dye and the fluorescent labels
10 each produce different emitting lights, the emitting lights each having a maximum
wavelength, distinguishable from each other, respectively, wherein
the maximum wavelength of the emitted light of the fluorescent analyte detection
dye differs from the maximum wavelengths of the emitted lights of each of the fluorescent
labels by at least 100 nm, and wherein the wavelengths of the first and second exciting
15 lights differ by at least 100 nm and one of the wavelengths of exciting lights is greater than
about 750 nm;
- d) detecting the emitted light of the first fluorescent analyte detection dye and the
emitted light of the first and second fluorescent labels; and
- e) correlating the detected emitted lights with the particle under analysis.

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24. The method of claim 23 comprising more than one particle, each particle having a
different fluorescent analyte detection dye, and two or more than two fluorescent labels in a
combination of relative amounts, wherein the combination of fluorescent labels in each
particle is different.

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25. The method of claim 23 comprising moving two or more than two particles through
an examination zone, each particle having a different size, the method further comprising

- f) directing the exciting light of the first wavelength at each particle in the
examination zone to generate a scattered light; and
- 30 g) detecting the scattered light; and

- h) correlating the detected scattered light with the emitted lights and the particle under analysis.

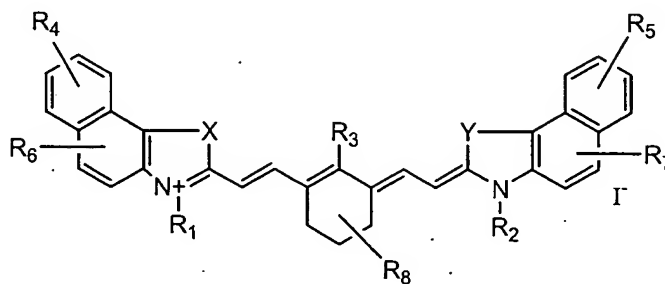
26. The method of claim 23 comprising moving two or more than two particles through an examination zone, the method further comprising

- f) directing an exciting light of a third wavelength at each particle in the examination zone to excite a fluorescent analyte detection dye, and
g) detecting the emitted light from the third exciting light; and
h) correlating the detected emitted lights with the particle under analysis.

27. The method of claim 26, each particle including a different size, the method further comprising:

- i) directing an exciting light at each particle in the examination zone to generate a scattered light; and
j) detecting the scattered light; and
k) correlating the detected scattered light with the emitted lights and the particle under analysis.

28. The use of a fluorescent label in a particle for detecting an analyte comprising a particle having a fluorescent label of the formula:



wherein:

X and Y are each independently selected from the group consisting of O, S, NR₉, and CR₉R₁₀;

R₁ and R₂ are each independently selected from the group consisting of H, C₁-C₂₀ alkyl, C₁-C₂₀ haloalkyl, C₁-C₂₀ alkylene, or C₁-C₂₀ haloalkylene;

5 R₃ is selected from the group consisting of H, halogen, OH, OR₁₁, SR₁₁, NR₁₁R₁₂, C₁-C₆ alkyl, C₁-C₆ alkylene, C₃-C₆ cycloalkyl, C₃-C₆ cycloheteroalkyl, C₃-C₆ cycloalkylene, C₃-C₆ cycloheteroalkylene, phenyl, biaryl, heteroaryl, or heterobiaryl, wherein the C₁-C₆ alkyl, C₁-C₆ alkylene, C₃-C₆ cycloalkyl, C₃-C₆ cycloheteroalkyl, C₃-C₆ cycloalkylene, C₃-C₆ cycloheteroalkylene, phenyl, biaryl, heteroaryl and heterobiaryl groups may be substituted with halogen, OH, C₁-C₄ alkyl, or C₁-C₄ haloalkyl;

10 R₄, R₅, R₆, and R₇ are each independently selected from the group consisting of halogen, OH, C₁-C₄ alkyl, or C₁-C₄ haloalkyl, phenyl, or heteroaryl, or other aromatic substituents known to those skilled in the art;

R₈ is selected from the group consisting of C₁-C₄ alkyl, or C₁-C₄ haloalkyl;

R₉ and R₁₀ are each independently selected from the group consisting of hydrogen, C₁-C₄ alkyl, or C₁-C₄ haloalkyl;

15 R₁₁ and R₁₂ are each independently selected from the group consisting of C₁-C₆ alkyl, C₃-C₆ cycloalkyl, phenyl, biaryl, heteroaryl, or heterobiaryl, wherein the C₁-C₆ alkyl, C₁-C₆ cycloalkyl, phenyl, biaryl, heteroaryl, and heterobiaryl groups may be substituted with halogen, OH, C₁-C₄ alkyl, or C₁-C₄ haloalkyl, or when R₃ represents NR₁₁R₁₂, R₁₁ and R₁₂ may be taken together to form an optionally substituted C₃-C₆ aliphatic or C₃-C₆ aromatic heterocyclic ring.

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